Interferon-gamma Release Assays: *the Good, the Bad, and the Ugly*

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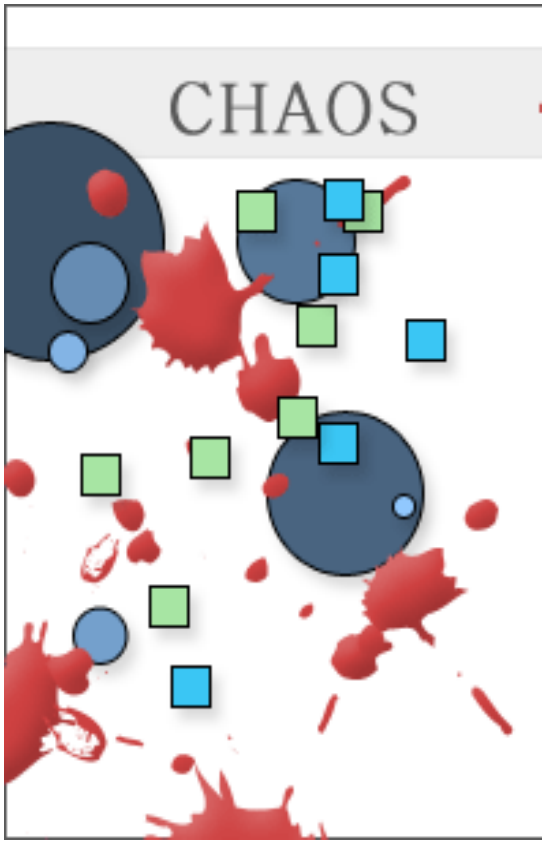
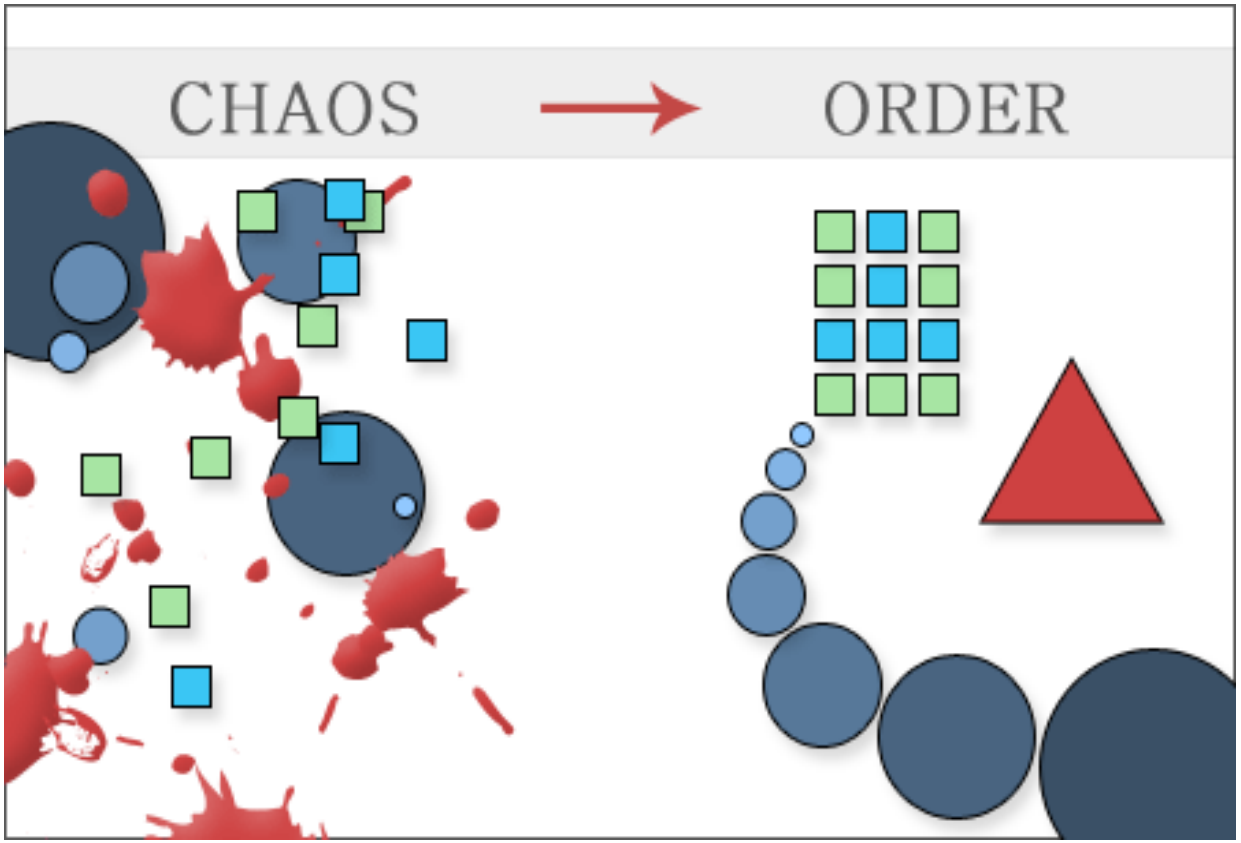
Disclosures with respect to this talk:

none

State of the field…



State of the field…



Not a comprehensive summary,

but rather an attempt to frame key issues

Topics

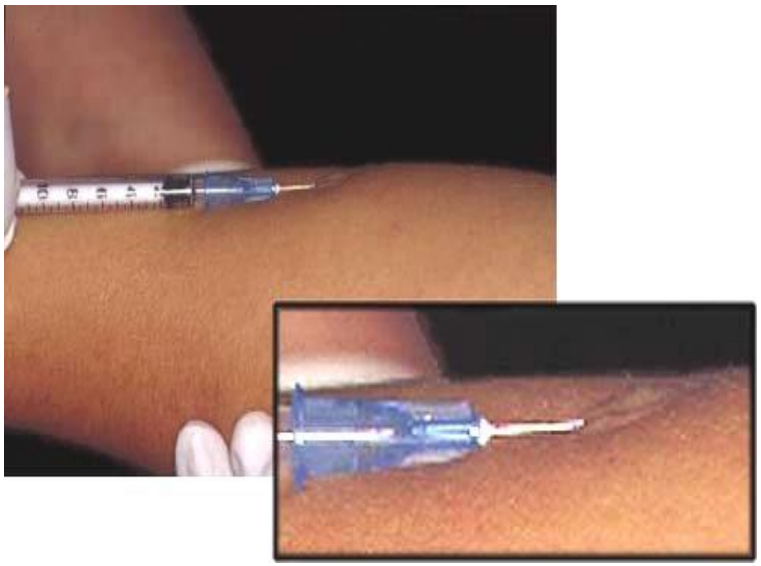
- IGRAs: what are they?
- How has their performance been assessed, in absence of a gold standard for latent TB?
- Sensitivity and specificity in adults
- Sensitivity and specificity in special populations
- Are IGRAs useful in monitoring treatment response?
- Serial testing, with a focus on healthcare workers

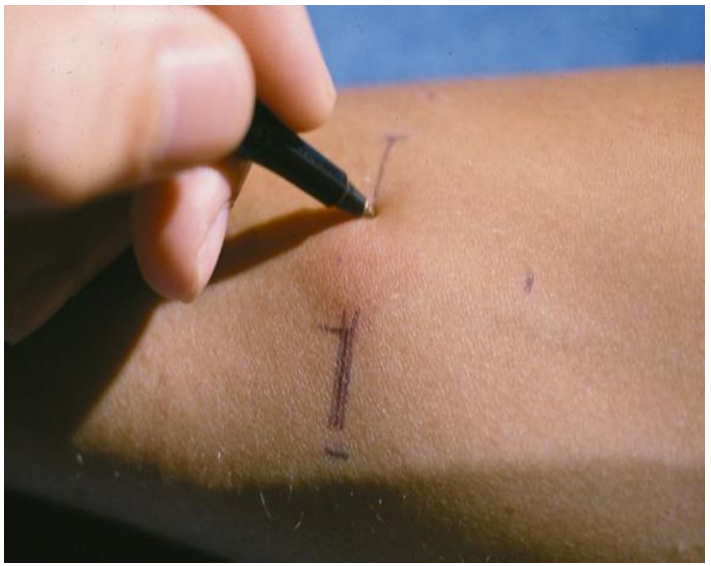
We want a ‘TB’ test to…

- Identify individuals who are infected with *M. tuberculosis*
- Identify infected individuals who will become sick with TB in the future
- Detect individuals who are currently sick with TB
- Inform whether TB treatment is working in an individual
- Inform whether a treated individual is cured

Tuberculin Skin Testing

- Intradermal inoculation of antigens (purified protein derivative)
- Local immunologic recognition of antigens (in previously sensitized persons)
- Local inflammation (“induration”) in previously sensitized persons





Tuberculin Skin Testing

- Merits
  - Relatively simple
  - Clinicians are accustomed to using, interpreting
  - Bedrock of U.S. TB control (operationally)
  - Benefits of preventive therapy for TST+ and absence of benefit of preventive therapy for TST- are proven

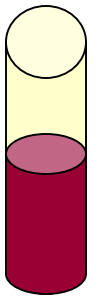
- Potential Problems
  - Not specific for *M. tuberculosis* (PPD=purified protein derivative)
  - 2 clinical interactions required to get test result
  - Interpretation somewhat subjective

CAN A BETTER TEST BE DEVELOPED ?

What about a BLOOD test?

- Avoids reader “subjectivity”
- A test result can be achieved after one patient-provider interaction
- Can use antigens other than PPD

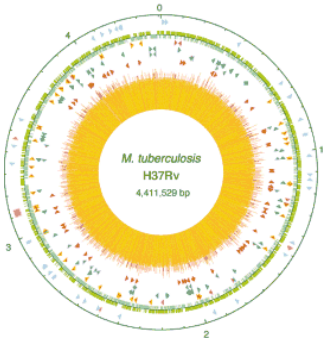
QuantiFERON®-TB Gold In-Tube (Qiagen)
T-SPOT.TB (Oxford Immunotec)



Stimulate blood cells with antigens

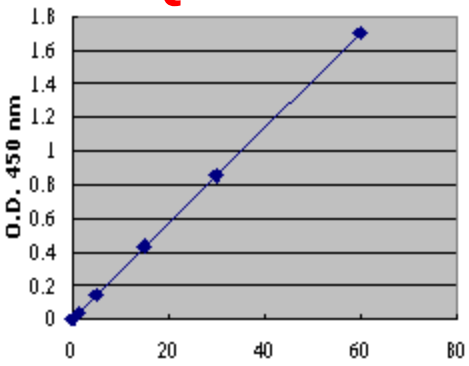
Incubate overnight

Detect IFNγ produced by cells

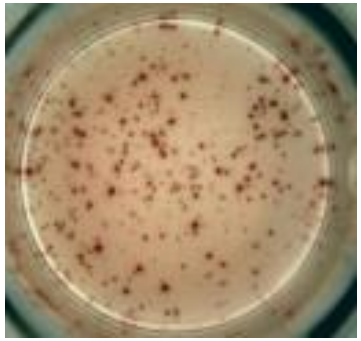


Use antigens SPECIFIC for *M. tuberculosis*: CFP10, ESAT6 (TB7.7 also in QFT-GIT)

QFT-GIT

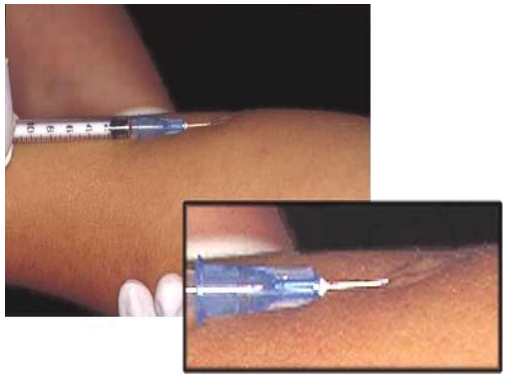


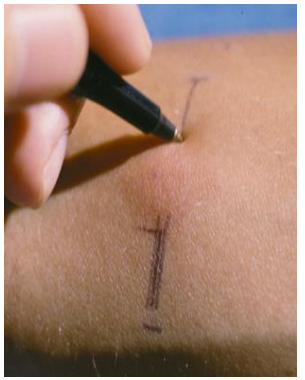
T-SPOT TB



Stimulate immune cells → Allow time for response by immune cells → Measure response

TST

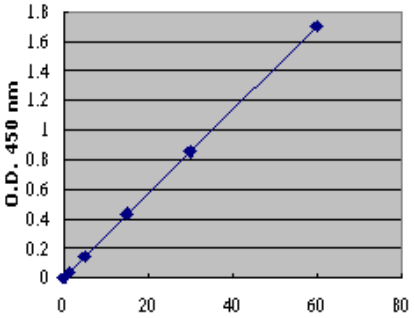




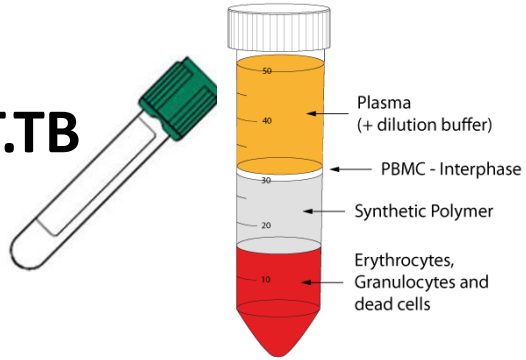
QFT-GIT

shake

LAB



T-SPOT.TB



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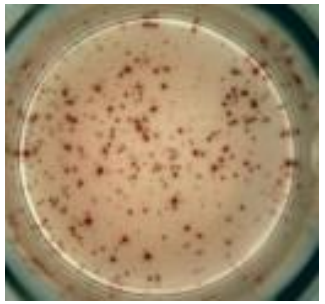
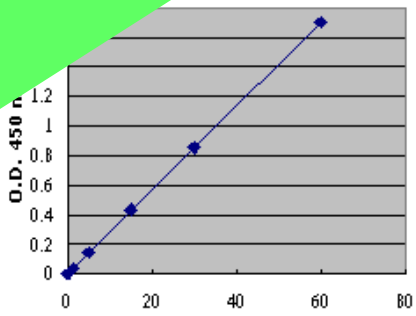


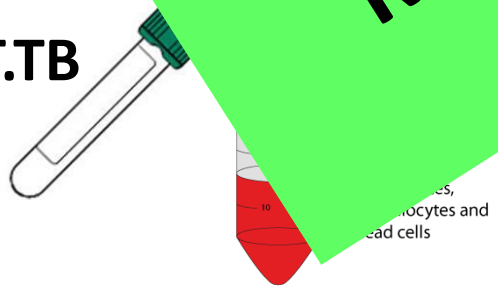
Stimulate immune cells → Allow ti… for re… → Measure response

TST

QFT-GI… shake



T-SPOT.TB



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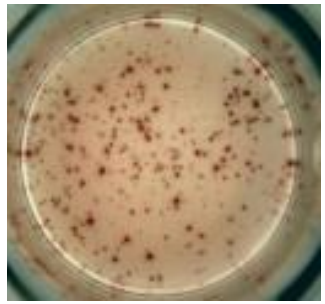

DIFFERENT METHODS: DIFFERENT PROS AND CONS

STRENGTHENING ONE ASPECT MAY EXPOSE WEAKNESS IN ANOTHER

Approaches to evaluating new tests for latent TB infection, in absence of true gold standard

- Direct comparison of TST results with results of new test
  - *Allows clinicians to relate performance of new test to that of a familiar test*
- Extrapolation based on evaluation of new test in people with active TB (SENSITIVITY)
- Determination of extent to which performance of new test fits a defined attribute (SPECIFICITY)
  - *Defined attribute is likelihood of latent TB infection based on clinical or epi characteristics, e.g. very low risk for Mtb infection*

These are not necessarily the target populations for a test for latent TB infection

Topics

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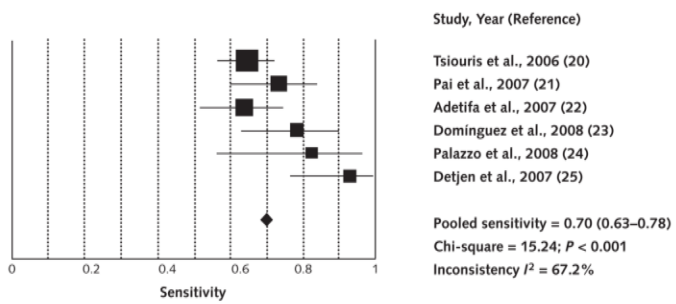
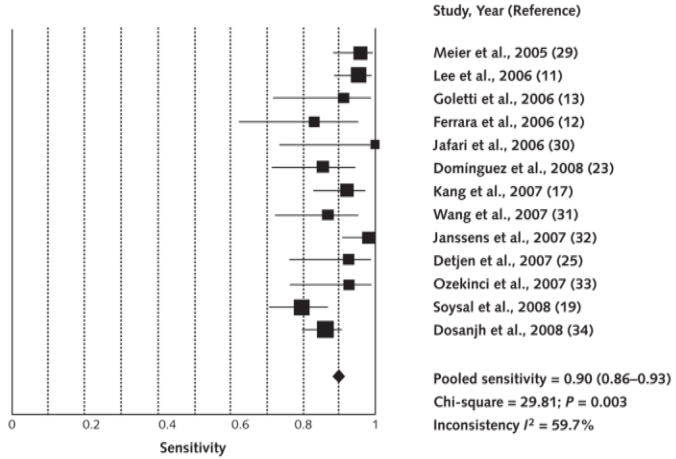
- Serial testing, with a focus on healthcare workers



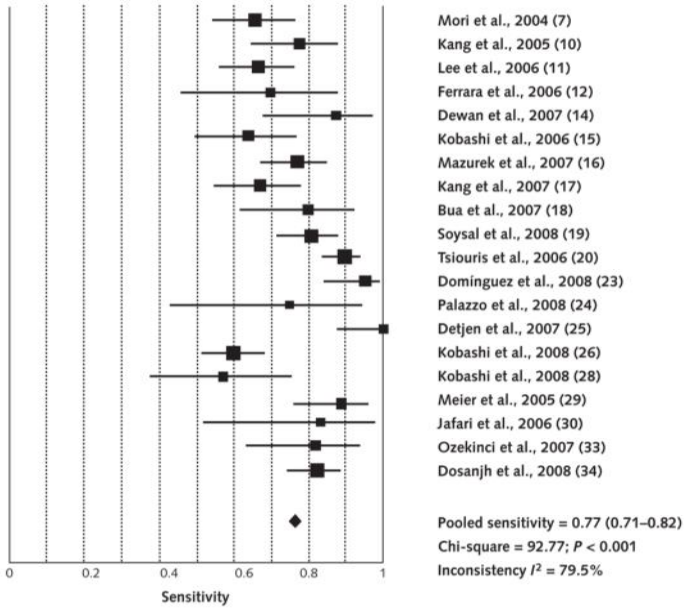
QFT-GIT
0.70 (0.63-0.78)

M. Pai et al. Ann Int Med 2008;149:177

(systematic review)



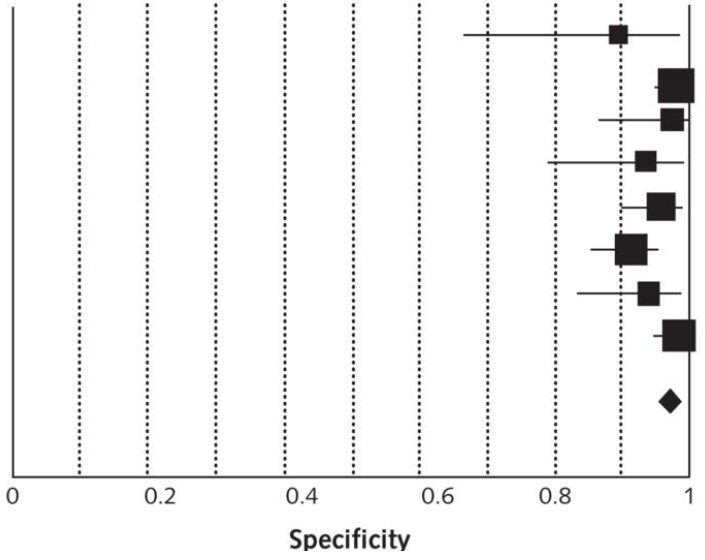
T-SPOT.TB
0.90 (0.86-0.93)



TST
0.77 (0.71-0.82)

In this series of studies, SENSITIVITY of T-SPOT.TB was slightly higher than that of QFT-GIT or TST

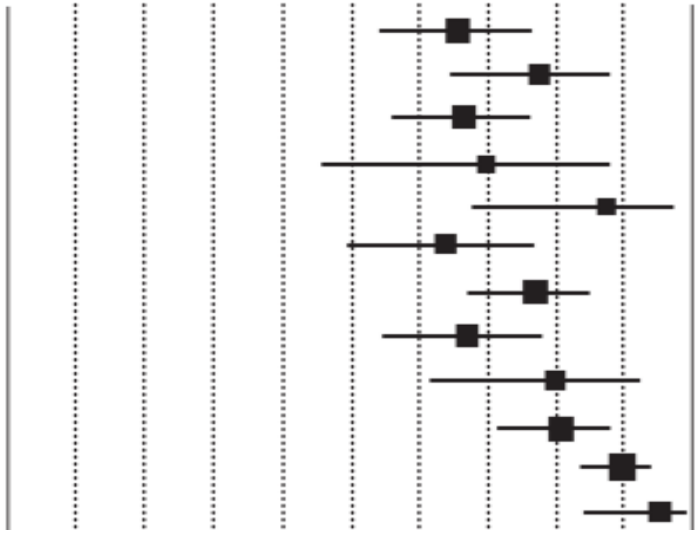
IGRAs and TST have high SPECIFICITY in non-BCG-vaccinated adults



Brock et al., 2001 (35)
Mori et al., 2004 (7)
Ravn et al., 2005 (9)
Brock et al., 2004 (36)
Kang et al., 2005 (10)
Lee et al., 2006 (11)
Kobashi et al., 2006 (15)
Soborg et al., 2007 (40)

Pooled specificity = 0.96 (0.94–0.98)
Chi-square = 13.81; $P = 0.055$
Inconsistency $I^2 = 49.3\%$

QFT
0.99 (0.98-1.00)



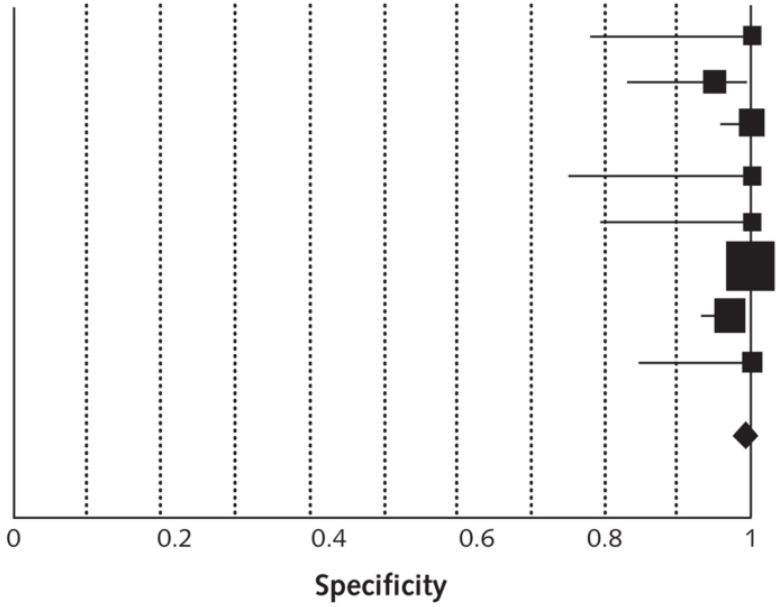
Study, Year (Reference)

Mori et al., 2004 (7)
Kang et al., 2005 (10)
Lee et al., 2006 (11)
Ferrara et al., 2006 (12)
Dewan et al., 2007 (14)
Kobashi et al., 2006 (15)
Mazurek et al., 2007 (16)
Kang et al., 2007 (17)
Bua et al., 2007 (18)
Soysal et al., 2008 (19)
Tsiouris et al., 2006 (20)
Domínguez et al., 2008 (23)

TST
0.97 (0.95-0.99)

M. Pai et al.
Ann Int Med
2008;149:177

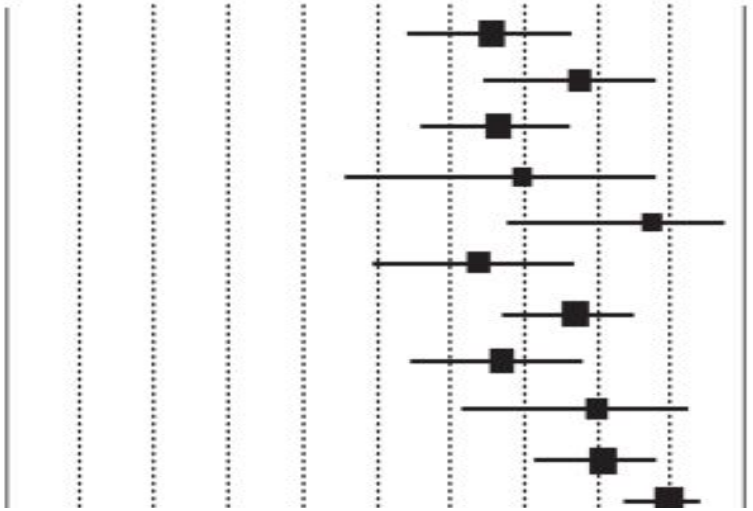
IGRAs **but not TST** retain high SPECIFICITY in BCG-vaccinated adults



Brock et al., 2001 (35)
Brock et al., 2004 (36)
Taggart et al., 2006 (37)
Palazzo et al., 2008 (24)
Bua et al., 2007 (18)
Mazurek et al., 2007 (38)
Franken et al., 2007 (39)
Detjen et al., 2007 (25)

Pooled specificity = 0.99 (0.98–1.00)
Chi-square = 15.88; $P = 0.026$
Inconsistency $I^2 = 55.9\%$

QFT
0.96 (0.94-0.98)



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Mazurek et al., 2007 (16)
Kang et al., 2007 (17)
Bua et al., 2007 (18)
Soysal et al., 2008 (19)
Tsiouris et al., 2006 (20)

TST
0.59 (0.46-0.73)

M. Pai et al.
Ann Int Med
2008;149:177

In adults:

The IGRAs, but not TST, retain specificity in BCG-vaccinated adults

sensitivity of T-SPOT.TB appears to be slightly higher than that of TST and QFT-GIT

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HIV-infected adults

JZ Metcalfe et al., JID 2011;204:S1120
(systematic review and meta-analysis of studies of adults in low- and middle-income countries)

		QFT-GIT	T-SPOT.TB
HIV-POS	Pooled SENSITIVITY in studies enrolling TB suspects	**60 (34-82)**	**76 (45-92)**
HIV-POS	Pooled SENSITIVITY in studies enrolling TB suspects and known active TB	**65 (52-77)**	**68 (56-80)**
HIV-NEG	Pooled SENSITIVITY in studies enrolling TB suspects and known active TB	**84 (78-91)**	**88 (81-95)**

head-to-head comparison of QFT-GIT and T-SPOT.TB:
3 studies, total n=36 HIV-positive adults with active TB
T-SPOT.TB sensitivity higher but not statistically significant

IGRAs in other immunosuppressed populations

- Autoimmune disease(s)
  - To date small studies, participants with variable immunosuppression or prior to immunosuppression
  - No compelling evidence base
- Transplant recipients
  - Paucity of data

IGRAs in Children

AM Mandalakas et al., IJTLD 2011;15:1018
systematic review and meta-analysis

	TST	QFT-GIT	T-SPOT.TB
Pooled **sensitivity** in children with active TB (95% CI)	80 (70-90)	83 (75-92)	84 (63-100)
Pooled **specificity** (95% CI)	85 (63-100)	91 (78-100)	94 (87-100)

For IGRAs, some studies have shown lower sensitivity in very young children – possible contributors are immunologic immaturity and challenges of making microbiological diagnosis of TB in very young children

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IGRA results “do not offer much value for treatment monitoring of TB disease”

Gamma Interferon Release Assay for Monitoring of Treatment Response for Active Tuberculosis: an Explosion in the Spaghetti Factory

Claudia M. Denkinger,[a,b] Madhukar Pai,[b,c] Meena Patel,[c] Dick Menzies[b,c]

JCM 2013;51:607

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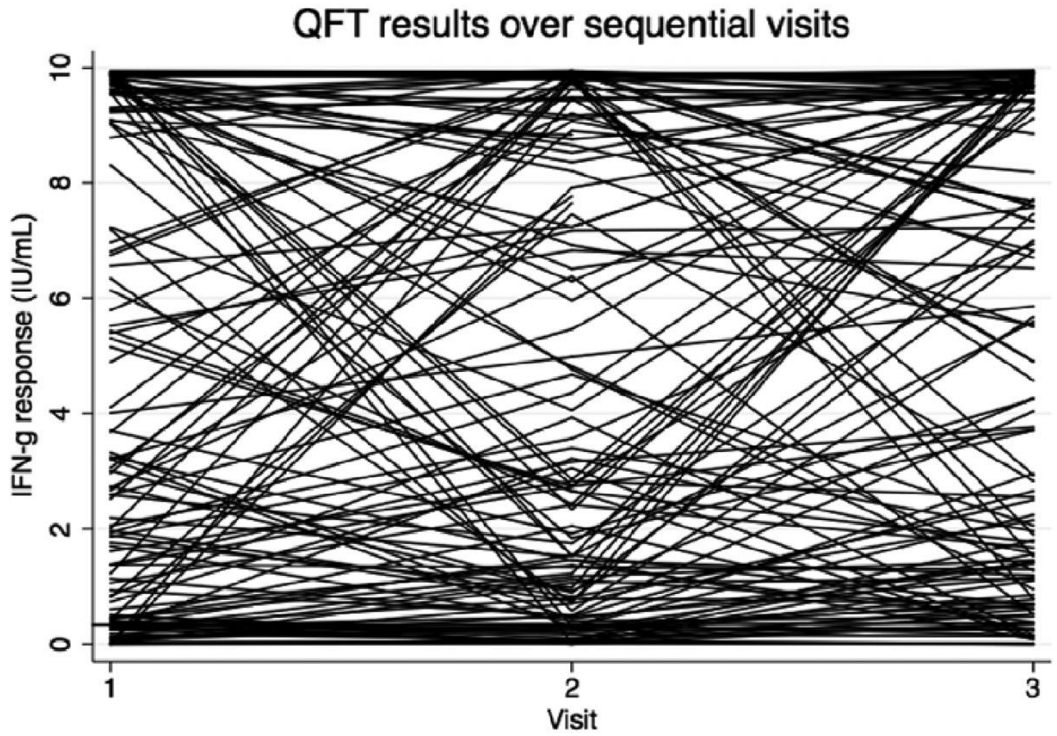
JCM 2013;51:607



128 pulm TB patients, DOT

No relationship between culture or smear status at 2 months and QFT-GIT dichotomous status or quantitative result

Overall, quantitative values declined during treatment

Large within-person variability on sequential testing

Topics

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CDC TB Epidemiologic Studies Consortium: IGRAs in HCWs ('TO-18')

- Longitudinal study of HCWs undergoing routine Occ. Health screening
- Large healthcare centers in 4 U.S. settings (Baltimore, Denver, NYC, Houston)
- QFT-GIT/T-SPOT.TB/TST every 6 months x 4

CDC TB Epidemiologic Studies Consortium: IGRAs in HCWs – Baseline Results

- 2495 enrolled (75% F, median age 36 y, 12% from high TB burden countries)
- 2122 (83%) completed 18 months of f/u

- Baseline positivity, by test

TST	5.2%	(125/2418)
QFT-GIT	4.9%	(118/2418)
T-SPOT.TB	6.0%	(144/2418)
All 3 tests	1.4%	(33/2418)

- Baseline pattern of TST pos / IGRA neg associated with prior BCG vaccination: OR 33.4 (95% CI 20-57)

CDC TB Epidemiologic Studies Consortium: IGRAs in HCWs – Conversions & Reversions

Baseline	% with status change among those retested	
TST POS (n=**125**)	54% (29/**54**)	Reversions differences not significant
QFT-GIT POS (n=118)	57% (67/118)	
T-SPOT.TB POS (n=144)	64% (92/144)	
TST NEG (n=2293)	0.9% (21/2293)	Conversions p<0.001 for QFT-GIT vs TST p=0.005 for T-SPOT.TB vs TST
TST NEG (n=2293)	1.2% (27/2293)*	
QFT-GIT NEG (n=2263)	6.1% (138/2263)	
T-SPOT.TB NEG or BL (n=2137)	8.3% (177/2137)	

* If TST conversion defined as dichotomous change from <10 mm to ≥10 mm

	Baseline result	Reversion	Conversion
QFT-GIT	**<0.01**		**5%** (52/1129)
	0.01-0.19		**7%** (65/972)
	0.20-0.35		**34%** (21/62)
	0.36-0.49	**97%** (28/29)	
	0.50-0.69	**70%** (16/23)	
	0.70-0.99	**54%** (7/13)	
	1.0-2.99	**52%** (12/23)	
	≥3.0	**13%** (4/30)	
T-SPOT.TB	**<1**		**4%** (54/1241)
	1-4		**13%** (92/727)
	5-7		**44%** (31/70)
	8	**77%** (13/17)	
	9	**84%** (16/19)	
	10	**75%** (6/8)	
	>10	**56%** (56/100)	

Among individuals with quantitative values just above or just below the cut-off threshold, 'reversions' or 'conversions' were common

	Baseline result	Reversion	Conversion
QFT-GIT	**<0.01**		**5%** (52/1129)
	0.01-0.19		**7%** (65/972)
	0.20-0.35		**34%** (21/62)
	0.36-0.49	**97%** (28/29)	
	0.50-0.69	**70%** (16/23)	
	0.70-0.99	**54%** (7/13)	
	1.0-2.99	**52%** (12/23)	
	≥3.0	**13%** (4/30)	
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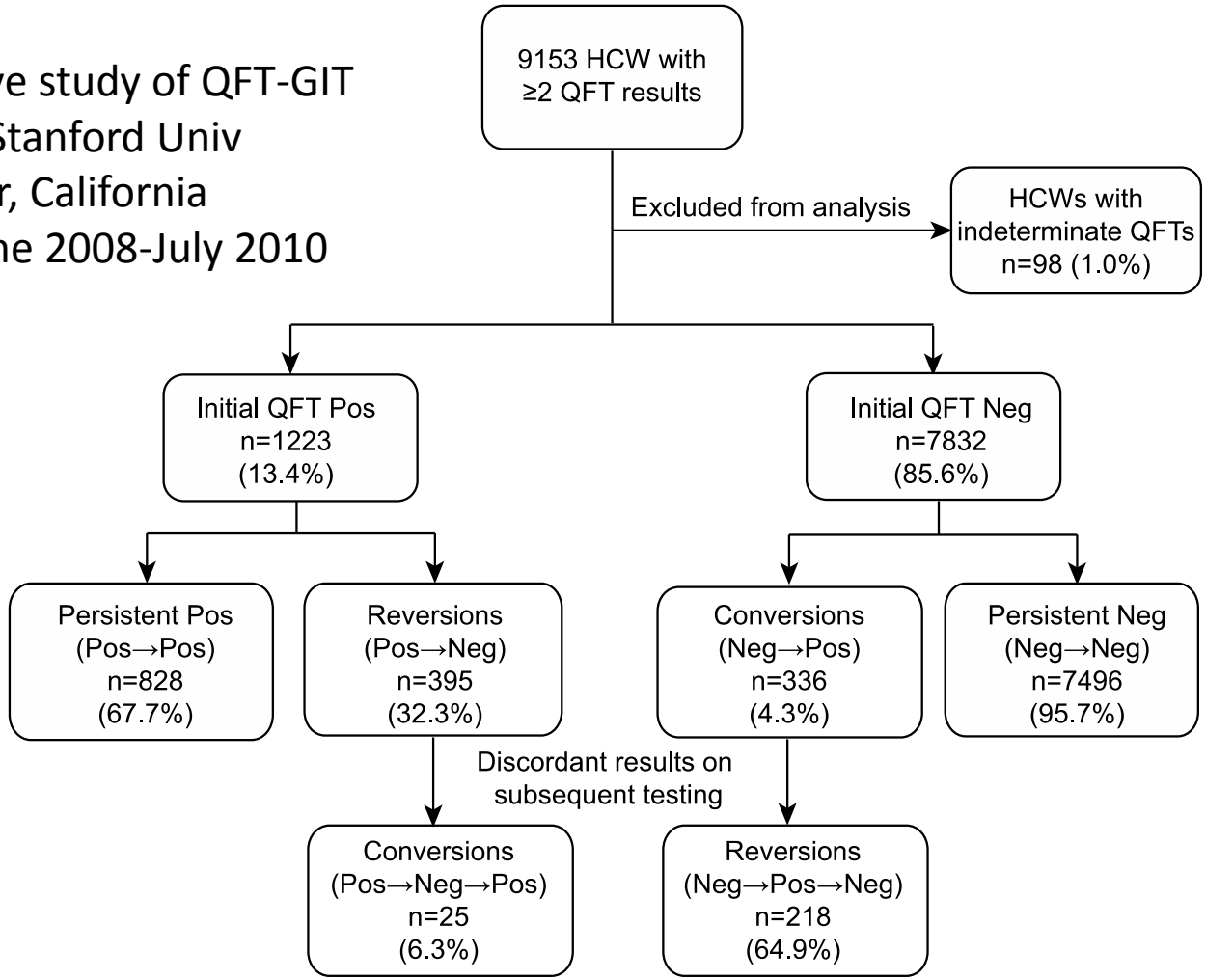
	10	**75%** (6/8)	
	>10	**56%** (56/100)	

Among individuals with quantitative values just above or just below the cut-off threshold, 'reversions' or 'conversions' were common

Another example:

ML Slater et al., AJRCCM on-line 26Aug2013

Retrospective study of QFT-GIT in HCWs at Stanford Univ Medical Cntr, California between June 2008-July 2010



Serial QFT-GIT testing of HCWs in North American settings: a non-systematic sampling

% conversion after initial neg	% reversion after initial pos	Notes	
4% (361/8227)	32% (395/1223)	Slater et al, AJRCCM 2013	Retrospective, routine practice
6% (138/2263)	57% (67/118)	Dorman/Daley et al, submitted	Cohort study
5% (13/245)	62% (8/13)	Zwerling et al, PLoSOne 2013	Cohort study
3% (164/6530)	49% (66/135)	Gandra et al, Inf Ctrl Hosp Epi 2010	Retrospective, routine practice
Not provided	40% (18/45)	Joshi et al, Can Respir J 2012	Retrospective, routine practice
3% (52/1857)	80% (8/10)	Fong et al, Chest 2012	Retrospective, routine practice

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Not provided	40% (18/45)	Joshi et al, Can Respir J 2012	Retrospective, routine practice
3% (52/1857)	80% (8/10)	Fong et al, Chest 2012	Retrospective, routine practice
7% (10/134)	33% (5/15)	Dorman/Daley et al: Sub-study: 2 weeks between tests	
6% (10/172) discordant		Dorman/Daley et al: Sub-study: 2 sets of tests drawn at once	

Potential sources of IGRA within-person variability that could impact sequential testing results

Person

Mtb exposure

Immune status
- HIV
- medications
- recent immunizations
- recent infections

Mtb antigen burden
Duration since exposure
Boosting from recent TST

Pre-analytical

Tube filling (QFT-GIT)
Tube shaking (QFT-GIT)
Tube storage (QFT-GIT)
Shipping (T-SPOT.TB)

Gaur et al, JCM 2013 epub
Median IFNg Ag:
Gentle shaking 0.12 IU/ml
Vigorous shaking 0.24 IU/ml

Ag tube 0.8 ml 1.04 IU/ml
Ag tube 1.0 ml 0.85 IU/ml
Ag tube 1.2 ml 0.49 IU/ml

Analytical

Reagent storage
Well-to-well x-contam
Other inconsistencies

Summary I

- TST, QFT-GIT, and T-SPOT.TB incorporate similar biological principals and all are ‘functional tests’
- Testing details/procedures differ; none is perfect, each has pros & cons
  - IGRAs have higher test completion rates than TST, since an IGRA result can be obtained in 1 encounter
- Assessing accuracy of a test for latent TB infection is challenging (what is ‘truth’?)
- Expansion of routine use of QFT-GIT and T-SPOT.TB has highlighted several challenges, esp related to serial testing

Summary II

- The biological specificity of IGRA test antigens provides an advantage for IGRAs over TST when testing BCG-vaccinated individuals
- Compared with QFT-GIT, T-SPOT.TB appears to have a slight sensitivity advantage and may be slightly less affected by immunosuppression
- IGRAs do not appear to have a role in monitoring treatment for TB (spaghetti)
- Unlike for TST, the benefit of preventive therapy in IGRA-positive individuals and lack of benefit in IGRA-negative individuals has not been proven
- For IGRAs used in serial testing of HCWs, rates of conversions and reversions (using simplistic definitions) are high and changes do not seem to reflect TB exposure; results are generally consistent across North American studies
  - The best way forward not entirely clear (change cut-points, repeat tests, etc) and may not be 'one-size-fits-all'
  - Heresy: maybe we should reconsider multiple aspects related to TB screening in North American HCWs

Conclusion

As for most medical tests, the decision to perform an IGRA or TST (at the patient or program level), and the action taken based on results…

should take into consideration test attributes including accuracy and feasibility, relevant epidemiology and patient clinical factors, and the goals of testing.

Thank you

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